ELECTROPHORESIS APPARATUS

Field Of The Invention

The present invention relates to an electrophoresis apparatus and methods of its use for fractionation of a complex sample.

Background Of The Invention

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed inventions, or that any publication specifically or implicitly referenced is prior art.

Electrophoresis has been widely applied in separating proteins, nucleic acids, and other charged molecule species for analytical or preparative purposes, and also in the analytical or preparative fractionation of heretogeneous populations of dispersed cells or other types of macroscopic particles. In the analysis of complex ampholytic samples, such as in proteomics, it would often be desirable to reduce the complexity of a sample by pre-fractionation. Two dimensional electrophoresis (2DE) is believed to be currently the most commonly used separation method in proteomics. In the first dimension of 2DE, conventional gel isoelectric focusing (CGIEF) or better yet, immobilized pH gradient IEF (IPGIEF) are used to separate proteins according to their pI values.

Both CGIEF and IPGIEF have numerous practical problems including a limited sample loading capacity, a limited dynamic range, precipitation of proteins during IEF

separation (streaking) and an inability to tolerate a large amount of salts in the samples. P.G. Righetti et al., (Electrophoresis 21, 2000, 3639-3648); P.G. Righetti et al., (Anal. Chem. 73, 2001, 320A-326A) and D.W. Speicher et al., (Anal. Biochem., 284, 2000, 266-278); X. Zou & D. W. Speicher, (Proteomics, 2, 2002, 58-68) have shown that pre-fractionation of a complex protein sample in a multi-compartmental electrolyzer significantly improves the performance of 2DE. It is believed that the common limitation of both the ISOELECTRIQ2™ unit, marketed by Proteome Systems™ and the ZOOM™ unit, marketed by INVITROGEN™ is two-fold. First, the distance between the center of the separation compartment and its walls is relatively large (greater than about 5 mm), and second, the electrophoretic migration distance in each compartment is long, about 25 mm and 13 mm, respectively. The first, coupled with the fact that the separation compartments are made of thermally insulating polymers, leads to poor Joule heat dissipation and severely limits the electric power that can be applied to the system (max. 5 W and 3.5 W, respectively). The second, coupled with the low electrophoretic mobilities brought about by the low field strength, a consequence of the limited heat dissipation capability of the systems and the long electrophoretic migration distance, leads to slow separation velocities. Consequently, the fractionation times in these systems are long, 6 to 16 hours and 4 hours, respectively. Both systems use compartments with relatively large volumes (about 5 ml and 0.7 ml for each compartment, respectively), and the volume of the compartments cannot be easily reduced.

Gradipore Limited (Life Therapeutics) developed a small scale electrophoresis unit for size-based and charge-sign-based fractionation of complex samples (WO 01/78878, incorporated herein by reference). It is believed that, in practice, active cooling of at least the electrolytes was required to prevent over-heating of labile proteins during electrophoresis. Gradipore subsequently developed a scaled-down version of the GRADIFLOW™ electrophoresis unit, for size-based and charge-sign-based pre-fractionation of complex samples. In this MICROFLOW™ system, about 3 cm x 4 cm polymer frames, separated by polyacrylamide membranes, are stacked next to each other to form the separation compartments and contain stagnant sample solutions. The compartment stack is terminated at both ends by a large volume anode compartment and cathode compartment. It is believed that in practice, the anolyte and catholyte are cooled and circulated through these compartments to provide convective heat removal.

Slow separation speed of the currently known electrophoresis systems, specifically, isoelectric fractionation systems, useful as they are, are believed to be due to the failure of existing systems to sufficiently address three interrelated design limitations. The first speed limitation comes from the fact that as the ampholytic components of a sample approach their isoelectric state, their electrophoretic mobilities approach zero. Consequently, when the components are close to their isoelectric state, they need an increasingly longer time to move across a certain distance. The second speed constraint comes from mechanical design problems that limit how short the electrophoretic migration path and how small the volume of the individual compartments holding the sample solutions can be before mechanical assembly and leak-tight sealing of the

compartments become very difficult. The third performance limitation comes from the amount of Joule heat that is produced during electrophoresis. Since Joule heat dissipation occurs through the walls of the separation compartment, and since heat must first be transported from the separation medium to the wall, both of which are inefficient processes, the amount of Joule heat produced during fractionation must be limited and external, active cooling means must be applied. This means that the electric power input into the system to effect a separation must be limited. This results in a low electric field strength which, in turn, results in slow electrophoretic migration velocities and concomitant long separation times presently observed with current apparatus.

Accordingly, there exists a need for an electrophoresis apparatus or device suitable for processing small volume samples while effectively dissipating the heat generated during electrophoresis and reducing separation times. More specifically, the second and third speed limitations discussed above can be eliminated or negated to a great extent by selecting a structural material for the separation compartments of an electrophoresis apparatus that is a good electrical insulator yet has a relatively high thermal conductivity and specific heat. From these materials, one could make separation compartments that act as high capacity heat sinks by creating small separation compartments with appropriately selected characteristic dimensions in relatively large pieces. These heat sinks would greatly mitigate the need for active external cooling and/or for the reduction of the electrophoretic power used.

Summary Of The Invention

The present invention relates to an electrophoresis apparatus and methods of its use for fractionation of a complex sample. The apparatus more specifically relates to

Membrane-Separated Wells for Isoelectric Focusing and Trapping (MSWIFT). Primary application areas of MSWIFT and its modes of operation are in the analytical-scale fractionation of complex samples, such as pre-fractionation of protein samples for proteomic analysis, preparation of fractions for mass spectral (MS) analysis, bioactivity testing, enzymatic analysis, etc., rapid selection of isoelectric membranes for preparative-scale isoelectric trapping (IET) separations, and characterization of isoelectric membranes.

The present invention provides for an electrophoresis apparatus for characterizing, measuring and/or altering a composition of a sample. The apparatus comprises an anode and a cathode, the cathode spaced from the anode so as to define a distance along a longitudinal axis, the anode and cathode further defining an electric field having a direction substantially along the longitudinal axis. The apparatus includes an anode compartment, the anode disposed therein and a cathode compartment, the cathode disposed therein. Each of the anode compartment and the cathode compartment can be configured to hold at least one electrolyte, and at least one of the anode compartment and the cathode compartment can be configured to hold at least a portion of the sample. Each of the anode compartment and the cathode compartment includes means for addition or removal of a solution, a first compartment dimension, a second compartment dimension, and a third compartment dimension. The first compartment dimension can be substantially orthogonal to the direction of the electric field, and the second compartment dimension can be substantially orthogonal to the direction of the electric field and the first compartment dimension. A ratio of the first compartment dimension and the second

compartment dimension defines an aspect ratio of the compartment, and the third compartment dimension can be substantially parallel to the direction of the electric field and substantially orthogonal to the first and second compartment dimensions. The apparatus further comprises an ion-permeable barrier positioned between the anode compartment and the cathode compartment. The ion-permeable barrier can be configured to prevent convective mixing between compartments. At least a portion of at least one of the anode and cathode compartments can be made of an electrically insulating material having a thermal conductivity greater than about 1 W/mK and a specific heat greater than about 100 J/kgK and the aspect ratio of at least one of the anode compartment and the cathode compartment can be less than one.

The electrophoresis apparatus preferably further comprises sealing means disposed between the anode compartment and the cathode compartment. The sealing means is preferably adapted to contain the ion-permeable barrier and provide access of ions to the ion-permeable barrier. Wherein the sealing means is made of a water insoluble polymer, the polymer can be natural or synthetic. Preferably, the water insoluble polymer of is selected from the group consisting of polyethylene, polypropylene, polyisobutylene, polyalkylenes, polyfluorocarbons, poly(dimethylsiloxane), poly(dialkylsiloxane), poly(alkylarylsiloxane), poly(diarylsiloxane), poly(ether ketones) or a combination thereof.

The electrophoresis apparatus further preferably comprises housing means for containing the anode and cathode compartments. Preferably, at least a portion of the housing means is made of a material having a thermal conductivity greater than about 1

W/mK and a specific heat greater than about 100 J/kgK. Moreover preferably, material of the at least portion of the housing means can be selected from the group consisting of alumina, aluminum nitride, zirconia, zirconium nitride, boron nitride, silicon nitride, silicon carbide, ceramics, fused silica, quartz, glass or any combination thereof.

The electrically insulating material of the at least one part of the anode or cathode compartment can be preferably selected from the group consisting of alumina, aluminum nitride, zirconia, zirconium nitride, boron nitride, silicon nitride, silicon carbide, ceramics, fused silica, quartz, glass or any combination thereof.

Preferably, the ion-permeable barrier is essentially free of weekly acidic functional groups or weakly basic functional groups or anionic functional groups or cationic functional groups. Alternatively, the ion-permeable barrier can be an isoelectric barrier.

In an alternative embodiment of an electrophoresis apparatus according to the present invention for measuring, characterizing, or altering a composition of a sample, the apparatus comprises an anode and a cathode, the cathode spaced from the anode so as to define a distance along a longitudinal axis, the anode and cathode further defining an electric field having a direction substantially along the longitudinal axis. The apparatus includes an anode compartment having an anode disposed therein and a cathode compartment having a cathode disposed therein. At least one separation compartment is preferably positioned between the anode and cathode compartments. Each of the anode compartment, cathode compartment and at least one separation compartment can be configured to hold at least one electrolyte. At least one of the anode compartment,

cathode compartment and at least one separation compartment can be configured to hold at least a portion of the sample, and each of the anode compartment, cathode compartment and at least one separation compartment includes means for an addition or removal of a solution, a first compartment dimension, a second compartment dimension, and a third compartment dimension. The first compartment dimension can be substantially orthogonal to the direction of the electric field, the second compartment dimension can be substantially orthogonal to the direction of the electric field and the first compartment dimension. A ratio of the first compartment dimension and the second compartment dimension defines an aspect ratio of the compartment, and the third compartment dimension is preferably substantially parallel to the direction of the electric field and substantially orthogonal to the first and second compartment dimensions. The apparatus further includes an ion-permeable barrier positioned between each of the anode compartment, the at least one separation compartment and the cathode compartment. The ion-permeable barrier can be configured to prevent convective mixing therebetween. At least a portion of at least one of the anode compartment, the cathode compartment and the at least one separation compartment is made of an electrically insulating material having a thermal conductivity greater than about 1 W/mK and a specific heat greater than about 100 J/kgK and the aspect ratio of at least one of the anode compartment, the cathode compartment and the at least one separation compartment is less than one.

The present invention further provides for a method of altering a composition of a sample by electrophoresis which includes providing an electrophoretic apparatus according to the present invention. The method further includes selecting an ion-

permeable barrier for use between the anode and cathode compartments, providing an electrolyte to the anode compartment, providing an electrolyte to the cathode compartment, providing at least a portion of a sample to at least one of the compartments, creating an electrophoretic direct current between the anode and the cathode by applying an electric potential between the anode and the cathode, and causing a transfer of at least one part of at least one component of the sample across the ion-permeable barrier. Alternatively, a method according to the present invention can include providing at least a portion of a sample to at least one of the compartments of an apparatus according to the present invention, providing at least one electrolyte to any of the compartments free of a sample component, creating an electrophoretic direct current between the anode and the cathode by applying an electric potential between the anode and the cathode, and causing a transfer of at least one part of at least one component across an ion-permeable barrier.

Brief Description of the Drawings

The accompanying drawings, which are incorporated herein and constitute part of this specification, illustrate an embodiment of the invention, and, together with the general description given above and the detailed description given below, serve to explain features of the invention.

FIGS. 1A and 1B are exploded top and plan cross-sectional views of a first preferred embodiment of an electrophoresis apparatus according to the present invention;

FIG. 2A and 2B are exploded top and plan cross-sectional views of another preferred embodiment of an electrophoresis apparatus according to the present invention;

FIG. 3A and 3B are exploded top and plan cross-sectional views of another preferred embodiment of an electrophoresis apparatus according to the present invention;

FIG. 4A is a top view of a preferred embodiment of an electrode compartment or separation compartment for use in an electrophoresis apparatus according to the present invention;

FIG. 4B is a cross-sectional view of the compartment of FIG. 4A along line IVB-IVB;

FIG. 4C is a plan view of the compartment of FIG. 4A along line IVC-IVC;

FIG. 5A is a top view of a preferred embodiment of an electrode compartment or separation compartment for use in the apparatus of FIG. 1;

FIG. 5B is a cross-sectional view of the compartment of FIG. 5A along line VB-VB;

FIG. 5C is a plan view of the compartment of FIG. 5A;

FIG 6A is a cross-sectional view of a preferred embodiment of sealing means for use in another preferred embodiment of an electrophoresis apparatus according to the present invention;

FIG 6B is a plan view of the sealing means of FIG. 6A;

FIG. 7A is a preferred embodiment of a sealing means for use in the apparatus of FIG. 1;

FIG. 7B is a cross-sectional view of the sealing means of FIG. 7A along line VIIB-VIIB;

FIG. 7C is a plan view of the sealing means of FIG. 7A;

FIG. 8 is a graphic result of an imaging isoelectric focusing (ICIEF) analysis of a sample processed by an electrophoresis apparatus according to the present invention;

FIG. 9 is a graphic result of an experiment for determining the isoelectric point of a membrane using an electrophoresis apparatus according to the present invention;

FIG. 10 is a graphic result of an imaging isoelectric focusing (ICIEF) analysis of fractions obtained from an egg-white sample using an electrophoresis apparatus according to the present invention;

FIG. 11 is a graphic result of a polyacrylamide gel IEF separation of a sample obtained using an electrophoresis apparatus according to the present invention;

FIG. 12 is another graphic result of another polyacrylamide gel IEF separation of a sample obtained using an electrophoresis apparatus according to the present invention.

Detailed Description

Shown in FIGS. 1A, 1B, 2A, 2B, 3A, and 3B, are preferred embodiments of an electrophoresis apparatus or device **10** for measuring, characterizing, and/or altering a composition of a sample. Device **10** can be used to fractionate a biological sample so as to add or remove at least a portion of a component from a sample solution. More specifically, device **10** can be used in the pre-fractionation of protein samples for proteomic analysis, the preparation of fractions for mass spectral analysis, bioactivity testing, enzymatic analysis and other applications focused on the isolation of components.

In the embodiment shown in FIGS. 1A and 1B, apparatus **10** includes a first element defining or forming anode compartment **14** and a second element defining or forming cathode compartment **15**, each of which can be individually inserted and axially spaced apart within housing means **1** along a longitudinal axis **A-A**. Anode and cathode compartments **14, 15** are each preferably configured to hold at least one electrolyte. In addition, either anode or cathode compartments **14, 15** of apparatus **10** can be further

configured to hold at least a portion of the sample to be altered. Each of anode and cathode compartments **14, 15** have means for adding or removing a solution to or from its respective compartment. As shown, anode and cathode compartments **14, 15** can be preferably configured so as to have an opening from the top thereby making anode and cathode compartment **14, 15** accessible for top loading or removal of a solution. Alternatively, anode and cathode compartments can be configured with other structures or alternatively located openings to provide access for adding or removing a solution from the compartments. Preferably respectively disposed within anode and cathode compartments **14, 15** are electrodes (not shown) acting as anode **30** (not shown) and cathode **35** (not shown). Anode and cathode **30, 35** are axially spaced apart substantially along longitudinal axis A-A by a distance **d** and can be further configured so as to provide an electric field having a direction **E** substantially parallel to longitudinal axis **A-A**. The electric field is applied for the purpose of performing the electrophoresis. Anode **30** and cathode **35** can be connected to a power source (not shown), more preferably, anode **30** and cathode **35** can be connected to a variable voltage source having a preferred voltage ranging from about 10 V to about 5000 V, with a current preferably ranging from about 0.01 mA to about 1000 mA. It is to be understood that either compartment **14** or **15** can act as the anode compartment and cathode compartment by connecting the appropriate outlet of the power source to the electrode in the respective compartment functioning as anode **30** and cathode **35**.

Preferably disposed between anode and cathode compartments **14, 15** and within housing means **1** can be one or more separation elements defining or forming separation

wells or compartments **40**. Although specifically shown in FIGS. 1A and 1B are first separation compartment **22** and second separation compartment **23**, it is to be understood that apparatus **10** can include as many separation compartments **40** as needed for a given electrophoresis application. Each of separation compartments **40**, including first and second separation compartments **22, 23** can be configured to hold at least one electrolyte and can be preferably further configured to hold at least a portion of the sample to be altered. Shown in FIGS. 2A and 2B is an alternative embodiment of apparatus **10'** having a single separation compartment, and shown in FIGS. 3A and 3B is yet another embodiment of apparatus **10''** having no separation compartment between anode and cathode compartments **14** and **15**.

Housing means **1** orients and seals anode compartment **14**, cathode compartment **15** and where provided, separation compartment **40** such that compartments **14, 15** and **40** are substantially aligned along longitudinal axis **A-A** so as to facilitate communication therebetween in which components of the solution to be altered can migrate between compartments **14, 15** and **40** under the influence of the electric field. In order to prevent fluid loss from compartments **14, 15** and **40** to the environment, apparatus **10** can further include sealing means **12**. Preferably, housing means **1** is configured so as to permit top loading of anode, cathode, and separation compartments **14, 15, 40** and sealing means **12** into housing means **1**.

Referring again to FIGS. 1A, 1B, 2A, 2B, 3A and 3B, sealing means **12** can be disposed about each of anode and cathode compartments **14, 15** and about separation compartments **40** where present. Each of sealing means **12** is preferably configured to

contain ion-permeable barrier **18**. Ion-permeable barrier **18** permits electrophoretic migration of selected ions from one compartment **14, 15, 40** to another while substantially restricting convective mixing of solutions contained in compartments **14, 15** and **40.**

In order to facilitate the sealing action of sealing means **12,** housing means **1** can include axially opposed compression members **8, 9,** preferably formed from an electrically insulating, non-brittle, sufficiently rigid material, such as PVC material, that can be axially displaced along longitudinal axis **A-A** to compress anode and cathode compartments **14, 15,** sealing means **12,** ion-permeable barriers **18,** and where present, separation compartments **40**. In addition, axial displacement of opposed compression members **8, 9** facilitates removal and/or replacement of the individual anode, cathode and separation compartments **14, 15, 40**, sealing means **12** and ion-permeable barriers **18** from housing means **1**. Compression members **8, 9** can directly act on axially opposed end plates **16, 11** which are each preferably engaged with sealing means **12** to transmit the compressive force to the assembled anode and cathode compartments **14, 15,** separation compartments **40**, sealing means **12** and ion-permeable barriers **18.** Compression members **8, 9** can include a threaded rod and nut assembly **5** so as to axially displace compression members **8, 9** along longitudinal axis **A-A**, however it is to be understood that other means of linear displacement may be provided.

Preferably, at least a portion of housing means **1** is made from a material having a thermal conductivity greater than about 1 W/mK, and a specific heat of greater than about 100 J/kgK, preferably greater than about 250 J/kgK, and especially greater than about 500 J/kgK. Referring to FIGS. 1A, 2A, and 3A housing means **1** can include insulating plate

2 preferably formed from alumina (not shown) and base plate 3 preferably formed from aluminum (not shown) and a cover (not shown). Preferably, anode, cathode and separation compartments **14, 15, 40** and sealing means **12** and ion-permeable barriers **18** are located on insulating plate **2** (not shown). Insulating plate **2** can electrically isolate base plate **3** from each of anode, cathode and separation compartments **14, 15, 40** and sealing means **12** and ion-permeable barriers **18**. Moreover, insulating plate **2** (made of alumina) can act as a heat sink during the electrophoresis operation of apparatus **10, 10'** and **10''**. More preferably, the material forming housing **1** can be alumina, aluminum nitride, zirconia, zirconium nitride, boron nitride, silicon nitride, silicon carbide, ceramics, fused silica, quartz, glass or other ceramic materials or any combination thereof. Moreover, base plate **3** (made of aluminum or stainless steel or other suitable metal) can also act as a heat sink during the electrophoresis operation of apparatus **10, 10'** and **10''**.

FIGS. 4A, 4B, and 4C and FIGS. 5A, 5B, and 5C are varying views of preferred embodiments of removable anode compartment **14** and cathode compartment **15** of apparatus **10, 10', 10''** and of separation compartment **40** of apparatus **10, 10'**. Anode, cathode and separation compartments **14, 15** and **40** can be formed from alumina, aluminum nitride, zirconia, zirconium nitride, boron nitride, silicon nitride, silicon carbide, ceramics, fused silica, quartz, glass or other ceramic materials or any combination thereof, so that heat generated during electrophoresis is dissipated to the structural material of compartments **14, 15** and **40** to insure that the components in the sample contained within compartments **14, 15** and **40** are not unduly heated. As a result,

the need to provide external active (forced) cooling of either the electrolyte or the sample solution can be greatly mitigated, and the electrophoretic power used to operate apparatus **10, 10'** and **10''** can be increased. The undesirable surface characteristics of alumina, zirconia, etc., (variable zeta potential, strong adsorptive binding of proteins) can be easily modified by post-manufacturing surface treatment well known in the art, such as by covalent or noncovalent binding of monomolecular layers or very thin films of protein-binding inhibitors, e.g., hydrophilic organic materials or polymers, onto the surfaces that are exposed to solutions. However, it should be understood that other electrical insulating materials having a relatively high thermal conductivity and specific heat can be used as well. More specifically, the material used to form any individual anode, cathode and separation compartment **14, 15** and **40** have heat transfer properties including a thermal conductivity higher than about 1 W/mK, preferably higher than about 10 W/mK, especially higher than about 20 W/mK and having a specific heat higher than about 100 J/kgK, preferably higher than about 250 J/kgK, and especially higher than about 500 J/kgK.

Specifically shown in FIGS. 4A, 4B and 4C is an illustrative embodiment of anode compartment **14** defined by first element of apparatus **10** as being a substantially circular cylindrical disk-like member. However, other geometries of the first element defining anode compartment **14** are possible, for example, as seen in the illustrative embodiment of FIGS. 5A, 5B and 5C, showing an alternative embodiment of the first element defining anode compartment **14'** as being substantially rectangular in cross-section. Shown more specifically in each of FIG. 4A is a top view of the first element

having an upper surface **17**. Anode, cathode and separation compartments **14, 15** and **40** can be preferably formed by a grinding operation, but other techniques are possible, for example, casting or molding. Alternatively, where a large number of anode, cathode and separation compartments **14, 15, 40** are to be produced from alumina, compartment **14, 15** or **40** can be formed prior to firing of the alumina.

As seen in FIGS. 4A, 4B, 4C and 5A, 5B, and 5C, anode compartment **14, 14'** is preferably accessible through upper surface **17** so as to permit top loading of a sample or electrolyte solution into compartment **14.** Referring to FIGS. 4A, 4B, 4C and 5A, 5B and 5C, anode compartment **14, 14'** is preferably defined by a width or first characteristic dimension **"a"** a depth or second characteristic dimension **"b"** and a length or third characteristic dimension **"c"**. First dimension **a** and second dimension **b** define a preferably substantially rectangular cross-section area **37** that is substantially orthogonal to longitudinal axis **A-A** when, for example, anode compartment **14** is inserted in housing **1**. However, other cross-sectional geometries are possible. Moreover, for example, when anode compartment **14** is inserted in housing **1**, first dimension **a** is preferably orthogonal to longitudinal axis A-A or the direction **E** of the electric field, second dimension **b** is preferably substantially orthogonal to both the first dimension **a** and the direction **E** of the electric field and third dimension **c** is preferably substantially parallel to the direction **E** of the electric field along longitudinal axis **A-A**. In addition, first and second dimensions **a, b** define an aspect ratio of anode compartment **14** as a ratio of first dimension **a** to second dimension **b**. First and second dimensions **a** and **b** are preferably selected such that the aspect ratio of anode compartment **14** is less than one. Preferably, the aspect ratio is less

than about ½, more preferably the aspect ratio is less than about 1/5, yet more preferably the aspect ratio is less than about 1/10 and even more preferably the aspect ratio is less than about 1/20.

It is to be understood that cathode compartment **15** and any number of separation compartments **40** of apparatus **10, 10'** or **10''** can be independently similarly or variably configured in a manner as described herein with respect to anode compartment **14**. More specifically, the aspect ratio of anode compartment **14** can be different from the aspect ratio of cathode compartment **15** and/or separation compartments **40** by varying first and second dimensions **a, b** of the respective compartments provided the aspect ratio of the respective compartments remains less than one. In a preferred embodiment, separation compartment **40** can be formed by grinding a 1.5 mm wide, 5 to 45 mm deep groove into 99.8% nonporous alumina blocks. Alternatively, grooves can be formed in alumina blocks as thin as 0.25 mm and as thick as 2.5 mm.

Preferably first dimension **a** and third dimension **c** are minimized. Minimizing first dimension **a** can in turn minimize the distance in the solution through which heat can be conducted to the wall of anode compartment **14,** cathode compartment **15** and/or separation compartment **40.** Minimizing third dimension **c** can mean that for a given applied potential, the electric field strength, and consequently the electrophoretic migration velocities of the components of the sample being processed are high, thus reducing the required separation time. Moreover, by minimizing third dimension **c**, i.e., the migration distance in a particular compartment, the overall distance from anode compartment **14** to cathode compartment **15** is minimized and therefore the separation

time in processing the sample is once again further reduced. For example, all else being equal, if one replaced a 9 mm I.D., 10 mm long cylindrical separation compartment (with a volume of approximately 636 µl) by a 2 mm by 2 mm by 154 mm rectangular well with a volume of approximately 616 µl (migration distance x width x height of the well), the separation time would decrease about 25-fold (five times due to the reduced migration distance and five times due to the five-fold higher electric field strength for a constant applied potential). Additional benefits would accrue from the smaller temperature rise in the separation well brought about by the smaller heat conduction distance (4.5 mm vs. 1 mm). Preferably, so as to facilitate minimization of first and third dimensions **a, c**, apparatus **10, 10', 10''** is preferably configured such that compartments, **14, 15, 40**, sealing means **12** and ion-permeable barriers **18** are substantially axially aligned within housing means **1**.

Again referring to FIGS. 4A, 4B, 4C and 5A, 5B, and 5C, shown is anode compartment **14, 14'** having first dimension **a**. First dimension **a** is preferably less than about 5 mm, more preferably less than about 3 mm, and even more preferably less than about 1 mm. The length of first dimension **a** can be varied, e.g., by the grinding operation forming anode compartment **14**. For example, forming compartment **14** using a jig with grinding wheels of different thickness allows for flexible changing of first dimension **a**. Preferably, as shown, walls **38, 42** are parallel with respect to one another. Alternatively, walls **38** and **42** can be tapered with respect to one another.

Third dimension **c** defines the migration distance of a component through anode compartment **14**, cathode compartment **15** or separation compartment **40**. Referring to

FIGS. 1A, 2A, and 3A, third dimension **c** of anode, cathode compartments **14, 15** and where applicable, any one of separation compartments **40** in apparatus **10, 10'** and **10''** can be either substantially equal or alternatively vary with respect to one another. Preferably, third dimension **c** of separation compartments **40** is less than about half the distance **d** between anode **30** and cathode **35.** More preferably, third dimension **c** of separation compartment **40** is about less than 1/3 the distance **d** between anode **30** and cathode **35.** An apparatus **10, 10'** having separation compartments with varying third dimensions **c** so as to vary the migration distances in the compartments, can provide flexibility in designing the shape of a pH gradient in the apparatus **10, 10'** and can further accommodate major components in larger volumes and minor components in smaller volumes. Moreover, the ability to have separation compartments **40** with varying third dimensions **c** can also provide a means to concentrate desired components into smaller volumes. Accordingly, where apparatus **10, 10'** can perform the electrophoresis process with partially filled wells or compartments **14, 15** and/or **40,** samples of widely different volumes can be handled in the same device.

Second dimension **b** or depth of the compartment permits the use of open (from the top) compartments **14, 15** or **40,** and provides for variable (partial) filling of compartments **14, 15** or **40** between zero and their respective full volume. There is no theoretical limit to the magnitude of second dimension **b** of the separation compartment orthogonal to the directions of both the electric field and first dimension of the compartment. Second dimension **b** can be varied to increase or decrease the required maximum reception volume of compartment **14, 15** or **40,** without degrading the

separation speed or the thermal characteristics of apparatus **10, 10', 10''**. Second dimension **b** can be varied by varying the dimensions of the material used to form compartment **14, 15** or **40**, in conjunction with control of the grinding operation forming compartment **14, 15** or **40.** Second dimension **b** can be as shallow as 5 mm and as deep as 40 mm. Shown in FIGS. 4C and 5C, first dimension **a** is defined by the distance between walls **38, 42** defining compartment **37.** Preferably as shown, walls **38, 42** are parallel with respect to one another. Alternatively, walls **38, 42** can be tapered with respect to one another.

First dimension **a,** second dimension **b** and third dimension **c** of each anode compartment **14,** cathode compartment **15,** and separation compartment **40** defines a reception volume for each to hold a volume of solution containing a sample component and/or an electrolyte. Preferably, anode and cathode compartment **14, 15** and where applicable, separation compartment **40,** of apparatus **10, 10', 10''** can receive a small volume of a solution containing an electrolyte and/or a sample component, the volume being less than about 5 ml, preferably less than about 2 ml, and more preferably between about 0.5 ml to about 0.001 ml.

In one alternative embodiment (not shown) of apparatus **10** shown in FIGS. 1A and 1B, apparatus **10** can include at least a first separation compartment **22** and at least a second separation compartment **23** having a reception volume greater than the reception volume of first separation compartment **22.** Preferably, the reception volume of anode compartment **14** and the reception volume of cathode compartment **15** are greater than the reception volumes of first and second separation compartments **22, 23.** In this

embodiment, walls **38, 42** of anode compartment **14** and cathode compartment **15** are preferably tapered relative to longitudinal axis A-A so as to produce a smooth transition between anode and cathode compartments **14, 15** to first and second separation compartments **22, 23**.

Referring again to FIGS. 1A, 1B, 2A, 2B, 3A, and 3B, apparatus **10, 10'** and **10''** can include sealing means **12** disposed about or in between each anode compartment **14**, cathode compartment **15** and where applicable, separation compartments **40** of apparatus **10, 10'** and **10''**. Shown in FIGS. 6A, 6B, 7A, 7B and 7C are preferred embodiments of sealing means **12**. In FIGS. 6A and 6B, sealing means **12** has a preferably substantially cylindrical disk shape, preferably made of silicone. Alternatively, sealing means **12** can be made from any water insoluble polymer, natural or synthetic, for example including, but not limited to, polyethylene, polypropylene, polyisobutylene, polyalkylenes, polyfluorocarbons, poly(dimethylsiloxane), poly(dialkylsiloxane), poly(alkylarylsiloxane), poly(diarylsiloxane), poly(ether ether ketones) or a combination thereof. Sealing means **12** further includes an opening **13** for providing an access through which ions present in a solution in anode and cathode compartments **14, 15** and where present, separation compartments **40** in apparatus **10, 10'** and **10''** can migrate to and access ion-permeable barrier **18**. Opening **13** is preferably substantially rectangular and includes a first characteristic dimension **a'** substantially corresponding to first dimension **a** of anode compartment **14**, cathode compartment **15** and where present, separation compartment **40**. Shown in FIGS. 1A, 2A, and 3A, preferably disposed between adjacent sealing means **12** are ion-permeable barriers **18**. Sealing means **12** can be configured to

position, locate or contain ion-permeable barrier **18** within opening **13** of sealing means **12.**

Other geometries of sealing means **12** are possible. A preferred alternative embodiment of sealing means **12** is shown in FIGS. 7A, 7B and 7C as **12'**. Sealing means **12'** is preferably formed from two silicone sheets joined together so as to form a pouch **19** for holding, containing and/or locating ion-permeable barrier **18.** Pouch **19** can further effectively eliminate or significantly reduce the wicking action of the membrane forming ion-permeable barrier **18.** Sealing members **12'** and pouch **19** are preferably formed by adhesively joining two silicone sheets together around a removable pouch-defining shim (not shown) or by polymerizing the silicon material around a removable pouch-defining shim (not shown) to form pouch **19.** The two silicone sheets used to form sealing means **12'** are preferably pre-cut 0.5 mm or 0.25 mm thick silicone sheets. Alternatively, sealing means **12'** can be made from any water insoluble polymer, natural or synthetic, for example including, but not limited to, polyethylene, polypropylene, polyisobutylene, polyalkylenes, polyfluorocarbons, poly(dimethylsiloxane), poly(dialkylsiloxane), poly(alkylarylsiloxane), poly(diarylsiloxane), poly(ether ether ketones) or a combination thereof. After sealing means **12'** is formed, the shim is removed leaving pouch **19** for location of ion-permeable barrier **18.** Pouch **19** is accessible from upper surface **21** of sealing means **12'** so that ion-permeable barrier **18** can be loaded into pouch **19** from the top of sealing means **12.'** Alternatively, ion-permeable barrier **18** can be completely encased in pouch **19** of sealing means **12'**, allowing it to communicate with its environment only through opening **13.** Alternatively,

sealing means **12'** can be cast or molded. Furthermore, the ability to create all seals at once, rather than one by one, reduces the required minimum structural distance in the direction of the electric field.

Sealing means **12'** includes an opening **13** for providing an access through which ions in a solution contained in anode compartment **14**, cathode compartment **15** and where present, separation compartment **40** can migrate to and access ion-permeable barrier **18**. Opening **13** is preferably substantially rectangular and includes a first characteristic dimension **a'** substantially corresponding to first dimension **a** of anode compartment **14**, cathode compartment **15** and where present, separation compartment **40.**

Ion-permeable barrier **18** facilitates alteration by electrophoresis of a composition of a sample contained in one or more of anode compartment **14**, cathode compartment **15** and separation compartment **40** of apparatus **10, 10', 10''** of FIGS. 1A, 2A, and 3A, respectively. Moreover, ion-permeable barrier **18** eliminates or mitigates convective mixing of the contents of adjacent anode, cathode and separation compartments **14, 15** and **40.** Ion-permeable barrier **18** can be a membrane having a defined pore size and pore size distribution for size-based and charge-sign-based electrophoretic separation of the sample components. Alternatively, ion-permeable barrier **18** can be an isoelectric membrane suitable for isoelectric trapping (IET) separations. Ion-permeable barrier **18** can also be configured so as to be essentially free of weekly acidic functional groups or weakly basic functional groups or anionic functional groups or cationic functional groups. Ion-permeable barrier **18** can also be configured to be an isoelectric membrane suitable

for isoelectric trapping (IET) separations and have a defined pore size and pore size distribution.

For size-based separations, ion-permeable barrier **18** is preferably made from polyacrylamide and preferably has a nominal molecular mass cut-off from about 1 kDa to 1500 kDa. The molecular mass cut-off of the membrane material selected for ion-permeable barrier **18** will depend on the sample being processed and the type of components in the sample.

For IET-based separations, at least one ion-permeable barrier **18** can be an isoelectric membrane formed from any suitable material. Examples include, but are not limited to, copolymers formed from acrylamide, bisacrylamide, acrylamido weak electrolytes and acrylamido strong electrolytes. Preferably, the membranes are thin or ultra-thin, having a thickness of about 2 mm or less, preferably about 1 mm or less, and especially about 0.2 mm or less. Where ion-permeable barrier **18** is an isoelectric membrane, barrier **18** is provided with a concentration of buffering species in the membrane material. The isoelectric membrane forming ion-permeable barrier **18** does not have to be thick to provide adequate buffering capacity. As long as the isoelectric membrane forming ion-permeable barrier **18** can mitigate convective mixing between the contents of adjacent compartments **14, 15** and **40**, the thinner the membrane, the shorter the distance the ampholytic components must travel. Therefore, thin isoelectric membranes can lead to shorter separation times. Also, for all else being equal, the thinner the isoelectric membrane, the less potential drops across it, and thus the less power is consumed to effect the electrophoretic separation. Additionally, most solutions used for

rehydration of the IPGIEF strips contain 0.1 - 1% carrier ampholytes. In IEF pre-fractionation of proteomics samples, the fractions typically do not contain a single isoelectric species with a single pI value, rather many components that cover a relatively wide pI range ($0.1 < pI < 2$). This means that in the fractions, even at the end of the separation, the carrier ampholyte and ampholytic sample molecules are typically not in their isoelectric state, but are protonated and deprotonated by each other. This also means that in these fractions the ionic strength is higher than at the end of an IET separation in which pure, single components are produced in a compartment. If, due to the improved heat dissipation performance of electrophoresis apparatus **10** one could add, in a sufficiently high concentration, carrier ampholytes or auxiliary isoelectric buffers to the sample prior to electrophoresis, one could significantly increase the ionic strength in the respective fractions. This would improve protein solubility and increase the total amount of material that can be loaded or processed in the given volume of the system.

The characteristics of ion-permeable barrier **18** used depend on the sample and the type of separation or treatment contemplated. Within a single apparatus **10, 10'** or **10''**, ion-permeable barriers **18** used may each be variably configured in a manner described herein to suit the electrophoresis application as needed. Ion-permeable barriers **18** or membranes can be purchased for use in the apparatus or made by the user prior to carrying out the desired electrophoresis run.

Referring again to FIGS. 1A, 2A and 3A, to assemble electrophoresis apparatus **10, 10'** and **10''**, anode and cathode compartments **14, 15** and where provided, separation compartments (wells) **40**, sealing means **12** and ion-permeable barriers **18** preferably

installed in pouches **19** can be placed into housing means **1** preferably from above. Once compartments **14, 15, 40**, sealing means **12** and ion-permeable barriers **18** are in place, wing nuts **5** are turned gently until they become finger tight over compression members **8,9** to create the seals. Compartments **14, 15, 40** are then filled with deionized water for a brief leak test. Once the system passes the leak test, anode and cathode compartments **14, 15** are filled with the respective anolyte and catholyte solutions, separation compartments **40** are preferably filled with the sample and where provided, an electrolyte, anode **30** and cathode **35** are respectively lowered into anode and cathode compartments **14, 15** and the electrophoretic potential is applied. In the case of an IET separation, the IET separation can be carried out using either constant potential, constant current or constant power input. Once the IET separation is complete (as indicated by the time course of the potential or the current), power is turned off and the contents of compartments **14, 15, 40** are removed for subsequent analysis or use.

In one exemplary assembled embodiment of an electrophoresis apparatus **10,** unfilled polycarbonate, for example, LEXAN® available from BOEDEKER PLASTICS, TX, is used to form housing means **1** and a ½ inch diameter borosilicate glass rod is used to form five separation compartments **40,** each having a holding volume of 50 µl. Ion-permeable barriers **18** are formed from isoelectric membranes which are installed between sealing means **12** formed from silicone disks which reduce solution loss from membrane wicking. Such an assembled apparatus **10** could be used for the separation of low molecular weight pI markers and proteins and for UV-active carrier ampholyte-based

membrane characterization. Using a surface treatment with a hydrophilic polymer on sealing means **12** can further reduce leaking problems and mitigate electroosmotic flow.

In another exemplary embodiment of an electrophoresis apparatus **10**, housing means **1** is preferably constructed from LEXAN® and seven separation compartments **40** are preferably formed from a 3/4 inch diameter borosilicate glass rod. Each separation compartment **40** defines a receiving volume of 150 µl. Ion-permeable barriers **18** are preferably isoelectric membranes installed in sealing means **12** including circular silicone pouches **19** that completely prevent liquid loss by wicking. Such an assembled apparatus **10** can be used for the separation of low molecular weight pI markers and proteins and for UV-active carrier ampholyte-based membrane characterization.

In another exemplary embodiment of an electrophoresis apparatus **10**, housing means **1** is preferably constructed from LEXAN® and six separation compartments **40** are preferably formed from rectangular ½ x ¼ x 1 inch, nonporous, 99.8% alumina blocks. Each separation compartment **40** defines a second dimension **b** of about 5 mm. Such an assembled apparatus **10** can be used for IET desalting, the separation of low molecular weight pI markers and proteins and for UV-active carrier ampholyte-based membrane characterization. Using a surface treatment with a hydrophilic polymer on sealing means **12** can further reduce leaking problems and practically eliminate electroosmotic flow.

In another exemplary embodiment of an electrophoresis apparatus **10**, housing means **1** is preferably constructed from LEXAN® and ten separation compartments **40** are preferably formed from rectangular, ½ x ¼ x 1 inch, nonporous, 99.8% alumina

blocks, each having a second dimension **b** being about 18 mm deep. Such an assembled apparatus **10** can be used for IET desalting, the separation of low molecular weight pI markers and proteins, for UV-active carrier ampholyte-based membrane characterization, and for the selection of the appropriate isoelectric membranes for larger scale membrane-based IET separations.

In yet another exemplary embodiment of an electrophoresis apparatus **10**, housing means **1** is preferably constructed from LEXAN® and twenty separation compartments **40** are preferably formed from rectangular, 2 x 35 x 55 mm, nonporous alumina blocks, each defining second dimension **b** as being about 40 mm deep. Such an assembled apparatus **10** can be used for IET desalting, the separation of low molecular weight pI markers and proteins, for UV-active carrier ampholyte-based membrane characterization and for the selection of the appropriate isoelectric membranes for larger scale membrane-based IET separations.

The method of altering a composition of a sample by electrophoresis using an apparatus **10, 10'** or **10''** includes selecting an ion-permeable barrier **18** for use between the anode and cathode compartments based upon the given application. Upon providing anode and cathode compartment **14, 15** with the requisite electrolyte, the sample can be added to one or more of compartments **14, 15** and **40**. Alternatively, using an apparatus 10, 10' or 10'', a sample can be added to one or more compartments **14, 15,** or **40** and an electrolyte can be added to any compartment **14, 15,** or **40** that does not contain the sample. Alternatively, using apparatus **10, 10',** or **10''** both a sample and an electrolyte can be added to one or more of compartments **14, 15,** or **40** and an electrolyte can be

added to any compartment **14, 15,** or **40** that does not contain the sample. Subsequently, an electrophoretic direct current between the anode and the cathode can be provided by applying an electric potential between the anode and the cathode so as to cause a part of a component of the sample being processed to transfer across an ion-permeable barrier **18**.

In a first preferred method of processing a sample using an electrophoresis apparatus **10, 10'** or **10''**, all ion-permeable barriers **18** inter-disposed in housing means **1** are preferably anti-convective isoelectric barriers. Selecting ion-permeable barriers **18** of this type produces fractions with predetermined pI ranges, *i.e.*, the system is operated in pure IET mode. The pI cuts can be as narrow or as broad as desired, depending on the characteristics of the sample and the objective of the electrophoretic separation, *i.e.*, prefractionation, selective component removal and/or enrichment of a component of the sample being processed. Fractionation can be achieved in the presence or absence of carrier ampholytes and auxiliary isoelectric buffers. This method of processing is especially flexible when compartments **14, 15** and **40** are variable within a single apparatus **10, 10', 10''** with respect to first characteristic dimension **a**.

In the second or alternative method of processing a sample using an electrophoresis apparatus **10, 10'** or **10''**, ion-permeable barriers **18** adjacent to anode compartment **14** and cathode compartment **15** are preferably anti-convective, isoelectric barriers. All other ion-permeable barriers **18** of apparatus **10, 10'** are preferably anti-convective, ion-permeable, non-isoelectric membranes. The fractions produced in the anode, cathode and/or separation compartments **14, 15, 40** still have distinct pI ranges. However, the respective pI ranges are not known a-priori, rather they depend on the

composition of the solution, *i.e.*, the relative amount of the carrier ampholytes, if used, the isoelectric auxiliary agent(s), if used, and the analytes (pure autofocusing mode). The advantage of this method or processing is that it allows for the production of fractions with pI ranges for which no isoelectric membranes are available. The drawback of this second method can be that the pI range boundaries associated with individual compartments **40** cannot be defined by the user ahead of the time. This second method of processing a sample is especially flexible when apparatus **10, 10'** includes a large number of separation compartments **40**, each with a very small third characteristic dimension **c**.

In another or third method of processing a sample using an electrophoresis apparatus **10** having at least two separation compartments **40**, ion-permeable barriers **18** adjacent to anode compartment **14** and cathode compartment **15** are preferably anti-convective isoelectric barriers, at least one ion-permeable barrier **18** inter-disposed between separation compartments **40** is preferably an anti-convective, isoelectric barrier, and at least one other ion-permeable barrier **18** is preferably an anti-convective, non-isoelectric barrier. Using this alternative method, the fractions produced in anode, cathode and/or separation compartments **14, 15, 40** also have distinct pI ranges: for some of them the pI range depends on the pI values of the isoelectric membranes delimiting the individual separation compartments **40**, for others it depends on the composition of the solution, i.e., the relative amount of the carrier ampholytes, if used, the isoelectric auxiliary agent(s), if used, and the analytes (mixed IET – autofocusing mode). This third or alternative method of processing a sample is advantageous when the pI boundaries for a major sample component are not known exactly, but one still would like to isolate

minor components with slightly lower and slightly higher pI values than the pI value of a major component. The drawback of the method is that the exact pI range boundaries of all the fractions cannot be defined by the user ahead of time. The third operation mode also benefits from the use of a relatively large number of separation compartments **40** having a very small third characteristic dimension **c**.

In yet another or fourth method of processing a sample using an electrophoresis apparatus **10, 10'**, ion-permeable barriers **18** adjacent to anode compartment **14** and cathode compartment **15** are preferably anti-convective, isoelectric barriers. The solutions in anode, cathode and separation compartments **14, 15, 40** can contain one or more isoelectric auxiliary agents. Additionally, at least one of ion-permeable barriers **18** of apparatus **10, 10'** is preferably an anti-convective barriers having a characteristic, size-dependent permeability. This alternative method of processing a sample allows for a size-based fractionation of components, especially when the amounts of sample components are relatively small compared to that of the isoelectric auxiliary agent(s) retained in the system by isoelectric trapping.

In another alternative or fifth method of processing a sample using an electrophoresis apparatus **10, 10', 10''** all ion-permeable barriers **18** are anti-convective and have a characteristic size-dependent permeability. At least one of anode, cathode and separation compartments **14, 15** and **40** can contain a solution of one or more isoelectric auxiliary agents. This method can be used for a rapid desalting of the sample or a size-based or charge-sign-based separation of its components. In a preferred method of desalting using the fifth method of processing a sample in apparatus **10, 10'**, the smaller

the number of separation compartments **40** provided, the faster the desalting, though the use of at least one separation compartment **40** adjacent to each of anode and cathode compartments **14, 15** might reduce the extent of protic shock for the sample components.

In yet another alternative or sixth method of processing a sample using an electrophoresis apparatus **10,** (known as a matrix deployment method), a plurality of separation compartments **40** and inter-disposed isoelectric ion-permeable barriers **18** ranging between a low and a high pI are provided, e.g., twelve separation compartments **40** and ten inter-disposed isoelectric ion-permeable barriers **18** ranging between a pI of 2 to a pI of 12 are provided, where the pI of each successive ion-permeable barrier **18** increases by 1.0. A complex biological sample, for example, a sample intended for proteomic analysis, is loaded into one or more of the ten separation compartments of first apparatus **10.** Anode and cathode compartments **14, 15** of first apparatus **10** are filled with an anolyte and catholyte, respectively. In this preferred method of use of electrophoresis apparatus **10,** the fractions produced in compartments **40** define the ten rows of a separation matrix.

After performing an IET separation for 10 to 30 minutes using first apparatus **10,** the content of each separation compartment **40** is transferred, preferably simultaneously, into ten separate apparatuses **10,** each having an anode compartment, a cathode compartment and ten separation compartments **40.** The ten apparatuses **10** in the second set of apparatuses define ten columns of the separation matrix. Accordingly, separation compartments **40** present in this second set of apparatuses **10** define the elements of the separation matrix. Ion-permeable barriers **18** adjacent to anode and cathode

compartments **14, 15** in apparatus **10** defining the columns of the separation matrix have the same pI values as ion-permeable barriers **18** inter-disposed between the respective separation compartments **40** of first apparatus **10** defining the rows of the separation matrix. Thus, e.g., ion-permeable barrier **18** between the anode compartment and the first separation compartment of apparatus **10** defining the first column of the separation matrix has a pI of 2, and ion-permeable barrier **18** between the cathode compartment and the last separation compartment of apparatus **10** defining the first column of the separation matrix has a pI of 3; ion-permeable barrier **18** between the anode compartment and the first separation compartment of apparatus **10** defining the second column of the separation matrix has a pI of 3, and ion-permeable barrier **18** between the cathode compartment and the last separation compartment of apparatus **10** defining the second column of the separation matrix has a pI of 4; ion-permeable barrier **18** between the anode compartment and the first separation compartment of apparatus **10** defining the third column of the separation matrix has a pI of 4, and ion-permeable barrier **18** between the cathode compartment and the first separation compartment of apparatus **10** defining the third column of the separation matrix has a pI of 5; etc. In each apparatus **10** defining the columns of the separation matrix, separation compartments **40** are isolated from each other by anti-convective, non-isoelectric ion-permeable barriers **18**. Thus, a temporally stable pH gradient is formed during the second electrophoretic separation across separation compartments **40** in each apparatus **10** defining the columns of the separation matrix, with the shape of the respective pH gradients depending on the relative amounts of the carrier ampholytes, where used, the auxiliary isoelectric buffers, where used, and

the sample constituents. Thus, separation compartments **40** in the first and second sets of apparatuses **10** define the elements of the separation matrix (10 x 10 = 100), and each respective separation compartment **40** contains fractions with a pI range of about 0.1. The resulting fractions can then be directly analyzed by mass spectroscopy, used for further research or digested and analyzed by mass spectrometry as common in proteomics to identify the constituent proteins.

If needed, the fractions can be subdivided further, preferably in another electrophoresis apparatus **10** wherein ion-permeable barriers **18** having a characteristic, size-dependent permeability are used in a manner substantially similar to the sixth method of processing a sample as described above. The resulting fractions can then be directly analyzed by mass spectroscopy, used for further research or digested and analyzed by mass spectrometry as common in proteomics to identify the constituent proteins. If needed, the respective digests can also be subjected to a subsequent IET separation in another apparatus **10** to provide fractions containing peptides with similar acidities. Such fractions are preferred for mass spectrometric analysis. This matrix operation mode provides a purely liquid-vein alternative 2DE-MS method for an analysis of the constituents of a complex, proteomic sample.

In an alternative method to the matrix deployment method, the content of each separation compartment **40** from the first IET separation is first stored, then sequentially transferred, ten times, into the ten separation compartments (wells) of the same, sequentially used electrophoresis apparatus **10**, and the IET analysis defining the columns

of the separation matrix is accomplished over a longer period of time, requiring only a single apparatus **10**.

In yet another alternative embodiment of the matrix deployment method described above, a first apparatus **10** having twenty-two separation compartments **40** is assembled using twenty inter-disposed isoelectric ion-permeable barriers **18** having pI values ranging between a pI of 2 to a pI of 12, where the pI of each successive ion-permeable barrier **18** increases by 0.5. A complex biological sample, for example, a sample intended for proteomic analysis, is loaded into one or more of the twenty separation compartments of first apparatus **10**. Anode and cathode compartments **14, 15** of first apparatus **10** are filled with an anolyte and catholyte, respectively. In this preferred method of use of electrophoresis apparatus **10,** the fractions produced in compartments **40** define the twenty rows of the separation matrix.

After performing an IET separation for 10 to 30 minutes using first apparatus **10,** the content of each separation compartment **40** is transferred, preferably simultaneously, into twenty separate apparatuses **10,** each having an anode compartment, a cathode compartment and twenty separation compartments **40**. This second set of electrophoretic devices, comprised of twenty apparatuses **10**, defines the columns of the separation matrix. Accordingly, separation compartments **40** present in this second set of apparatuses **10** define the elements of the separation matrix. Ion-permeable barriers **18** adjacent to anode and cathode compartments **14, 15** in apparatus **10** defining the columns of the separation matrix have the same pI values as ion-permeable barriers **18** inter-disposed between the respective separation compartments of first apparatus **10** defining

the rows of the separation matrix. Thus, e.g., ion-permeable barrier **18** between the anode compartment and the first separation compartment of apparatus **10** defining the first column of the separation matrix has a pI of 2, and ion-permeable barrier **18** between the cathode compartment and the last separation compartment of apparatus **10** defining the first column of the separation matrix has a pI of 2.5; ion-permeable barrier **18** between the anode compartment and the first separation compartment of apparatus **10** defining the second column of the separation matrix has a pI of 2.5, and ion-permeable barrier **18** between the cathode compartment and the last separation compartment of apparatus **10** defining the second column of the separation matrix has a pI of 3; ion-permeable barrier **18** between the anode compartment and the first separation compartment of apparatus **10** defining the third column of the separation matrix has a pI of 3.0, and ion-permeable barrier **18** between the cathode compartment and the first separation compartment of apparatus **10** defining the third column of the separation matrix has a pI of 3.5; etc. In each apparatus **10** defining the columns of the separation matrix, separation compartments **40** are isolated from each other by anti-convective, non-isoelectric ion-permeable barriers **18**. Thus, a temporally stable pH gradient is formed during the second electrophoretic separation across separation compartments **40** in each apparatus **10** defining the columns of the separation matrix, with the shape of the respective pH gradients depending on the relative amounts of the carrier ampholytes, where used, the auxiliary isoelectric buffers, where used, and the sample constituents. Thus, separation compartments **40** in the first and second sets of apparatuses **10** define the elements of the separation matrix (20 x 20 = 400), and each respective separation compartment **40**

contains fractions with a pI range of about 0.025. The resulting fractions can then be directly analyzed by mass spectroscopy, used for further research or digested and analyzed by mass spectrometry as common in proteomics to identify the constituent proteins.

If needed, the fractions can be subdivided further, preferably in another electrophoresis apparatus **10** wherein ion-permeable barriers **18** having a characteristic, size-dependent permeability are used in a manner substantially similar to the method of processing a sample as described above. Due to the fine pI resolution, the number of size-based fractions required might be relatively low (e.g., 4 to 6). The resulting fractions can then be directly analyzed by mass spectroscopy, used for further research or digested and analyzed by mass spectrometry as common in proteomics to identify the constituent proteins. If needed, the respective digests can also be subjected to a subsequent IET separation in another apparatus **10** to provide fractions containing peptides with similar acidities. Such fractions are preferred for mass spectrometric analysis. This high resolution matrix operation mode can provide a purely liquid-vein alternative to the 2DE-MS analysis of the constituents of a complex, proteomic sample and is believed to be just as (or more) powerful as the currently used prefractionation-2DE-MS methods, while being more suitable for robotics-based automation.

Another or seventh method of processing a sample includes using an electrophoresis apparatus **10** in which dilute samples or fractions can be concentrated by IET. A preferred apparatus **10** is assembled using separation compartments **40**, more specifically, a first separation compartment **22** and at least a second separation

compartment **23** smaller that the first separation compartment **22,** each compartment disposed between anode compartment **14** and cathode compartment **15**. Larger separation compartment **22** is preferably located adjacent to anode or cathode compartment **14, 15** (or both, if two larger separation compartments **22** are used). Preferably, anode and cathode compartments **14, 15** are each relatively large as compared to first and at least second compartments **22, 23**. Walls **37, 42** of first separation compartment **22** are preferably tapered, producing a smooth transition between anode and cathode compartments **14, 15** having preferably wider first dimensions **a** and second separation compartment **23** having preferably narrower first dimension **a**. To provide adequate potential drop across separation compartments **22, 23**, at least one isoelectric buffer is added to the sample to be fractionated. The pI value of the added isoelectric buffer is selected such that the isoelectric buffer is trapped in first separation compartment **22,** between isoelectric ion-permeable barriers **18** separating anode and cathode compartments **14, 15** and first separation compartment **22,** and isoelectric ion-permeable barrier **18** separating the large volume wells and at least one second smaller separation compartment **23**. In another preferred embodiment, simultaneous concentration and fractionation can be achieved using a plurality of separation compartments **40** separated by isoelectric or non-isoelectric ion-permeable barriers **18**.

EXAMPLES

Example 1: Fractionation of low molecular weight pI markers

An electrophoresis apparatus was assembled using six alumina elements that each contain a 40 x 2 x 2.5 mm compartment. The anode, cathode and four separation compartments were separated by five ion-permeable barriers made from isoelectric

membranes respectively having pI values of: pI = 2, pI = 3, pI = 5, pI = 6.5, and pI = 9.5. The anode compartment was filled with 60 mM methanesulfonic acid, and the cathode compartment was filled with a mixture of 20 mM lysine and 20 mM arginine. The separation compartment delimited by ion-permeable barriers of pI = 2 and pI = 3 contained 50 mM IDA. Nominal 200 µl aliquots of a sample containing 2% Pharmalyte 3 < pI < 10 carrier ampholytes and three pI markers: nicotinic acid (pI = 3.2), 4-hydroxy-2-(morpholinomethylene)-benzoic acid (pI = 5.8) and epinephrine (pI = 9.2) were loaded into each of the separation compartments of the apparatus. The power supply was operated at a constant power of 4 W for 14 min, yielding an initial potential of 213 V, final potential of 575 V, initial current of 16 mA and final current of 7 mA. The separation took a total of 121 Vh.

The content of each well was analyzed by the iCE280 ICIEF system (Convergent Bioscience, Toronto, Canada) before the IET separation and after the IET separation. The respective volume changes, the component peak areas and their ratios are set out in Table 1.

Table 1

Well	pI range	Vol change (µl)	Marker	$Area_{init}$	$Area_{final}$	Ratio
1	pI < 2	+5				
2	2 < pI < 3	0				
3	2 < pI < 3	-5	Nic	16490	44630	2.71
4	5 < pI < 6.5	+5	Morph	4412	12625	2.86

5	6.5< pI < 9.5	-5	Epi	20966	57278	2.73
6	9.5 < pI	0				

The results of an ICIEF run are shown in FIG. 8. Clearly, the UV-absorbing pI markers were completely moved in 14 min into the wells limited by the appropriate isoelectric membranes.

Example 2: Characterization of the pI of an isoelectric separation membrane

An electrophoresis apparatus was assembled using four alumina elements that each contain a 40 x 2 x 2.5 mm separation compartment. The anode, cathode and two separation compartments were isolated by three ion-permeable barriers made from isoelectric membranes, the first of which had a pI value of 2 , the second one was the membrane to be tested, and the third one was a membrane with a pI value of 11.5. The anode compartment was filled with 60 mM methanesulfonic acid and the cathode compartment was filled with 60 mM NaOH. Nominal 200 µl aliquots of a sample containing 2% Pharmalyte 3 < pI < 10 carrier ampholytes and 0.1% UV active carrier ampholytes were loaded into the two separation compartments. The power supply was operated at a constant power of 6 W for 15 min. After IET, the contents of the well adjacent to the anode compartment and the cathode compartment were analyzed by ICIEF using the iCE280 unit. The results are shown in Figure 9. Clearly, the carrier ampholytes were separated into two fractions indicating that the pI of the isoelectric membrane to be tested was 7.5.

Example 3: Fractionation of an egg-white sample

An electrophoresis apparatus was assembled using five alumina elements that each contain a 40 x 2 x 2.5 mm alumina compartment. The anode, cathode and three separation compartments were isolated by ion-permeable barriers made from isoelectric membranes respectively having pI values of: pI = 4; pI = 5.6, pI = 8.5 and a pI = 12. The anode compartment was filled with 50 mM IDA and the cathode compartment was filled with 60 mM NaOH. Nominal 200 µl aliquots of filtered egg white dissolved in 2% Pharmalyte 3 < pI < 10 carrier ampholytes were loaded into each of the three separation compartments. The power supply was operated at a constant potential of 500 V for 18 min, yielding a final current of 4 mA. After IET separation, the content of each compartment was analyzed by the iCE280 ICIEF system.

The results are shown in FIG. 10. The top panel is the ICIEF result for the pI markers, the second panel is the egg white feed sample mixed with the pI markers, the third panel is for the 4 < pI < 5.6 fraction, the fourth panel is for the 5.6 < pI < 8.5 fraction, and the fifth panel is for the 8.5 < pI < 12 fraction. The major proteins in each fraction (ovalbumin, ovotransferrin and lysozyme) reach their final destination well in as short a separation time as 18 min.

Example 4: Binary fractionation of a calf liver lysate sample

An electrophoresis apparatus was assembled using four alumina elements that each contain a 40 x 2 x 2.5 mm compartment. The anode, cathode and two separation compartments were separated by three ion-permeable barriers respectively having pI values of: pI = 5, pI = 6.5 and pI = 9.5. The anode compartment was filled with 60 mM CH_3SO_3H and the cathode compartment was filled with a mixture of 20 mM lysine and 20 mM arginine. Nominal 200 µl aliquots of 0.5 mg/ml calf liver lysate (7 M urea, 2 M

thiourea, 4% CHAPS, 3% 3 < pI < 10 Pharmalyte carrier ampholytes) were loaded into each of the three separation compartments. The power supply was operated at a constant power of 4 W for 15 min, yielding a final current of 5 mA. After IET separation, the content of each compartment was analyzed by IEF using 3 < pI < 10 IEF gels (Invitrogen). Results of the separation are shown in FIG. 11. There was a very sharp cut between the two protein fractions indicating that the IET separation was complete in as little as 15 min.

Example 5: Fractionation of a calf liver lysate sample

An electrophoresis apparatus was assembled using five alumina elements that each contain a 40 x 2 x 2.5 mm compartment. The anode, cathode and three separation compartments were separated by four ion-permeable barriers made from isoelectric membranes respectively having pI values of: pI = 3, pI = 5, pI = 6.5 and pI = 9.5. The anode compartment was filled with 60 mM CH_3SO_3H and the cathode compartment was filled with a mixture of 20 mM lysine and 20 mM arginine. Nominal 200 µl aliquots of 0.5 mg/ml calf liver lysate (7 M urea, 2 M thiourea, 4% CHAPS, 3% 3 < pI < 10 Pharmalyte carrier ampholytes) were loaded into a single separation compartment delimited by ion-permeable barriers of pI = 3 and pI = 5. The power supply was operated at a constant power of 4 W for 15 min, yielding a final current of 5 mA. After IET separation, the content of each well was analyzed by IEF using 3 < pI < 10 IEF gels (Invitrogen). Results of the separation are shown in Figure 12. There was a very sharp cut between the three protein fractions indicating that the IET separation was complete in as little as 25 min.

The electrophoresis apparatus described herein addresses many of the disadvantages of currently used isoelectric pre-fractionation apparatuses and methods such as the inability to tolerate high electric power loads, the need for active cooling, slow separation speeds, inconvenient system set-up and sample handling, and relatively large sample volumes that cannot be varied easily. The apparatus described herein may be used to separate varying volumes of complex samples into multiple fractions, with direct recovery of the fractions for subsequent analytical or biological characterization, in 10 to 30 min, using 5 to 10 W power, without active (forced) external cooling.

While the present invention has been disclosed with reference to certain embodiments, numerous modifications, alterations, and changes to the described embodiments are possible without departing from the sphere and scope of the present invention, as defined in the appended claims. Accordingly, it is intended that the present invention not be limited to the described embodiments, but that it have the full scope defined by the language of the following claims, and equivalents thereof.